Research Article
Hittite Journal of Science and Engineering, 2023, 10 (2) 125–134
ISSN NUMBER: 2148–4171
DOI: 10.17350/HJSE19030000299

Physicochemical Analysis of Pomegranate Sours Produced by Traditional Method in Türkiye and The Investigation of Antioxidant Properties

Mirac Oguz, Bulent Akar, Cemalettin Baltaci
Gumushane University, Department of Food Engineering, Gumushane, Türkiye

ABSTRACT

In this study, the physicochemical properties and antioxidant activities of the commercially produced 15 pomegranate sour samples and one control sample were determined and their compliance with the TS 12720 (traditional sour pomegranate concentrate standard) was evaluated. Antioxidant activity values of pomegranate sours extracts were determined using 6 different methods. The samples had strong antioxidant capacity, except for N10 and N15. In addition, glucose, fructose, sucrose, HMF and acidity measurements of the same samples did not show compatibility with TS 12720. The pH values of %37.5 of the all samples were not in accordance with the standard values (2.4-4.0). In addition, the measured titratable acidity values were below the standard value (>6.0% (m/m)) in 43.75 of the samples. While the brix values of the samples were measured between 59.20-75.70, the brix values of the % 18.75 of the samples were determined below the standard brix value (>68%). The highest HMF value of the samples were determined as 8117.66. According to TSE 12720, the HMF content should be not exceed 50 mg/kg. However, the HMF values of the samples were detected above the maximum limit value except for N8 and N16.

Keywords:
Pomegranate sour, Punica granatum, Antioxidant activity, TS 12720, Physicochemical

Article History:
Received: 2022/11/30
Accepted: 2023/05/23
Online: 2023/06/30

***Correspondence to**: Bulent Akar,*
Gumushane University, Food Engineering,
29000, Gumushane, TÜRKİYE
E-Mail: akarblnt@gmail.com;
Phone: +90 456 233 10 00;
Fax: +90 456 233 10 75.

This article has been checked for similarity.

INTRODUCTION

The main reason why herbal products have recently been demanded by consumers is their phenolic substances, which have a positive effect on health (1). Fruits and vegetables are rich in antioxidants that protect cells against oxidation (2). Plant products show high antioxidant activity because of that they include phenolic compounds (carotenoids, anthocyanidins and flavonoids) (3). Particularly, fruits have rich polyphenolic compounds which have significantly higher antioxidant activity than essential vitamins (4). Pomegranate is one of the fruits with high antioxidant activity. The pomegranate fruit is unique and rich in bioactive compounds and even has antioxidant activity as strong as BHT standard (5). Therefore, pomegranate fruit is included in a group called the superfruit, which has excellent nutritional quality and important chemicals for health (6). Therefore, as in the world, the consumption of pomegranate fruit and its products (pomegranate sour, pomegranate sauce and pomegranate molasses) have increased in Türkiye. As a matter of fact, the gradual increase in pomegranate cultivation in Türkiye confirms this situation (7).

The pomegranate (*Punica granatum* L.), which is in the Punicaceae family, originates from Central Asia (particularly Iran) and is distributed to other parts of the world (8). It is estimated that there are about in total 300.000 hectares of pomegranate cultivated area in the world and 76% of the total area is represented by 5 countries (India, Iran, China, Türkiye, and the USA) (6). A large number of food products (wine, jelly, jam etc.) are produced in pomegranate fruit and one of them is pomegranate sour. Pomegranate sour, which contains vitamins and beneficial chemical compounds, is a pomegranate product consumed in Türkiye. Pomegranate sour is obtained by caramelizing the sugar in pomegranate juice and evaporating water (9). However, some negative situations may occur during the production of pomegranate sour. For example, hydroxymethyl furfural (HMF) which is not in the natural structure of the pomegranate sour may be formed the during applied heat treatments. HMF consist of dehydration of sugar and its reaction with amino acids (10). In addition, these products can be adulterated with improper manufacture and storage conditions. For these reasons, it is necessary to perform studies to determine the content

Cite as:
Oguz M, Akar B, Baltaci C. Physicochemical analysis of pomegranate sours produced by traditional method in Türkiye and the investigation of antioxidant properties. Hittite J Sci Eng 2023; 10(2):125-134. doi:10.17350/hjse19030000299

of pomegranate products (11-12). In addition, studies have been carried out to determine the HMF content and some physicochemical properties of pomegranate sours produced in Türkiye. However, a limited number of pomegranate sour samples were analyzed in these studies; While İncedayı (11) used two different pomegranate sour samples for content analyzes Turkmen (13) analyzed the product belonging to a single sample with the control sample.

Therefore, main goal of the study is to determine the physicochemical properties and antioxidant activities of the commercially produced numerous pomegranate sour samples (15 different brands) and one control sample and compliance of these analyzes results with traditional sour pomegranate concentrate standard (TS 12720) of the Turkish Standardization Institute (14).

MATERIAL AND METHODS

Sampling

Traditionally produced pomegranate sours samples (15 samples in total, each belonging to a different company) were purchased from market at the 2020 years. The purchased pomegranate sours were in original glass bottles with label information and were selected among from their closer production dates each other. In addition to these samples, a pomegranate sour (control sample) was produced at the Gümüşhane University Food Engineering Laboratory to compared with other samples. All of the samples were stored in a dark place under room conditions. In order to the prepare the pomegranate sour, 1000 mL of pomegranate juice having %15.3±0.25 brix was transferred to a volumetric flask and the water was removed in the rotary evaporator (Heidolph Hei-Vap Value G1 Rotary Evaporator) at a pressure of 100 mbar, at the 60°C until the water-soluble solids were at least 72 brix during 3 h. The samples were kept at room temperature until analysis time. All of the analyses for each sample were performed as three replicates. All chemicals and solvents (analytical purity or HPLC purity) were purchased from Sigma-Aldrich (St.Louis.MO.USA) and Merck (Darmstadt, Germany).

Physical and Chemical Parameters

Color analysis

Hunter Colorimeter (Minolta CR-300 Colorimeter, Minolta Camera Co., Osaka, Japan) was used to measure the color values (L*, a*, b* and ΔE* (Equation 1)) of the samples. The a* value refers to the redness, the b* value refers to its yellowness and the L* value refers to the degree of light between 0 and 100 (black and white) of the food (15).

$$\begin{aligned} &\Delta E(\text{The total color difference}) = \sqrt{(\Delta L^2 + \Delta a^2 + \Delta b^2)} \\ &\Delta L \ : light - dark\ difference \ \left(L - L_0\right) \\ &\Delta a \ : red - green\ difference \ \left(a - a_0\right) \\ &\Delta b \ : yellow - blue\ difference \ \left(b - b_0\right) \end{aligned} \quad (1)$$

Identification of artificial food colors

To determine the presence of artificial food dyes in the samples, 40 g of the samples were diluted with 1-3 times with distilled water and then filtered. Concentrated HCl was dropped on the mixture as a few drops and sheep wool (oil removed) was immersed in the solution. After the solution was kept in a water bath for 1 hour, the sheep wool was washed. If the sheep wool was not dyed or dyestuff on it was removed by washing, it was concluded that there was no dyestuff in the sample. If the dye on the wool was not removed with washing, distilled water and a few drops of 5% NH_3 were added on it. Then, the mixture was boiled in a water bath for 30 min until the NH_3 evaporates. As a result of this process, it was concluded that if the dye in the wool diffused into the solution, it was artificially dyed otherwise natural dyed (16).

Rheological analysis

Flow behavior analyses of the pomegranate sour samples at different temperature were determined using a rheometer device (Anton Paar MCR 102, Thermo Scientific, Germany). The pomegranate sour samples were placed on the rheometer plate (diameter 35 mm, spacing 1.000 mm) at constant temperature (15, 25 and 35°C) to draw flow behavior graphs. The graphs were obtained with measuring shear stress in the range of 0-100 s^{-1} shear velocity. The visible viscosity values of the samples were determined at a shear rate of 50 s^{-1} based on the obtained data and the graphics (17).

Water soluble dry matter contents (Brix%)

Brix values of pomegranate sour samples were analyzed using an ABBE Refractometer (Optic Ivymen System, Spain). The pomegranate sour sample was placed between the prisms of the refractometer and closed. The water connections of the device were established and the temperature of the area where the sample placed in the device was set as 20°C (18).The optical refractive index value of the sample was read.

Titratable acidity

Titratable acidity (in citric acid) values of pomegranate sour samples were measured based on titration method (with monitoring pH) and it was calculated in anhydro-

us citric acid (%w/w) (19). Firstly, 10 grams of the sample were completed with 100 ml of distilled water. Then it was titrated with 0.1 N NaOH solution until the pH value reaches 8.3 and the calculations were based on the added amount of NaOH on the solutions (Equation 2).

$$\%Titratable\,acidity = \frac{VxNx0.064x1000}{m}$$
$$V: Volume\ of\ sodium\ hydroxidevolume\ (mL)$$
$$N: Normality\ of\ sodium\ hydroxide$$
$$m: Mass\ of\ the\ sample \quad (2)$$
$$0.064: Equivalent\ factor\ used\ to\ express\ acidity\ in\ citric\ acid$$

pH

pH of samples was measured using pH meter (Ohaus). The sample was shaken until homogeneous. Then the pH meter was calibrated using buffer solutions pH 4.0 and pH 7.0 and the device electrode was inserted into the homogenized pomegranate sour sample in the beaker and all of the measurements were performed.

5-Hydroxymethylfurfural (HMF)

5-Hydroxymethylfurfural (HMF) content of the samples was determined based on TS 6178 ISO 7466 (Determination of the content of fruit and vegetable products 5-Hydroxymethylfurfural) (20) which is modified Baltacı et al. (21). Pomegranate sour sample was weighted as 2.5 g and 25 mL distilled water was added onto it. To prevent deterioration of HMF, 0.25 mL Carrez I and 0.25 mL Carrez II solutions were transferred into mixture. Then it was completed 100 ml with distilled water. The solution was transferred into vials by passing through a 0.45 micron filter and injected into the conditioned HPLC system (Agilent 1200 series HPLC, Agilent Technologies, Palo Alto, CA, USA). The following parameters were selected for analytical column and elution solution in HPLC device.

Mobile phase: Water-methanol (90:10 volumetrically)
Flow Rate: 1mL/min
Wavelength: 285nm

The amounts of HMF in the sample were calculated based on the peak areas of the standard and sample solutions as mg/kg (Equation 3).

$$HMF\,\frac{mg}{kg} = \frac{V1}{M}\times\frac{1}{V2}\times\frac{(y-bo)}{m}$$
$$V1: HMF\ completed\ volume\ (mL)\ from\ 2.5\ g\ sample$$
$$V2: Solution\ (mL)\ injected\ into\ HPLC \quad (3)$$
$$M: Mass\ of\ the\ sample\ (g)$$
$$(y-bo)/m: Calibration\ constant$$

Fructose, glucose, sucrose and total sugar

Analyzes of total sugar, glucose, fructose and sucrose of the samples were performed (22). Firstly, 2.5 g of the sample was dissolved in 40 mL of distilled water and transferred into test tube having 25 mL methanol. The mixture was filtered and transferred into vials. Calibration solutions of glucose, fructose and sucrose standards were prepared at different concentrations and analyzed under the same conditions. The equation defining the curve was calculated with the linear regression analysis applied to the data. The following parameters were selected for analytical column and elution solution in HPLC device.

Flow rate: 1.3 mL/min
Mobile phase: Volumetrically acetonitrile/water (80:20)
Column temperature: 30°C ± 1°C
Injection volume: 20 µL

The peaks were identified for all standards and samples. Area and heights of the peaks were measured. It was drawn a linear calibration graph showing the concentrations of the standard (micrograms in milliliter) corresponding to peak areas. The response factor (RF) was obtained from a selected point on the calibration graph using a data acquisition/calculation system. Total sugar contents of the samples were determined using the Eq. 4.

$$Glucose, fructose, sucrose = \frac{V1}{M}\times\frac{1}{V2}\times\frac{100}{1000}\times\frac{(y-bo)}{m} \quad (4)$$

Antioxidant Activity

DPPH radical scavenging activity

The method developed by Brand-Williams et al. (23) is based on the reduction of a stable purple-colored compound DPPH radical, which shows strong absorption at a wavelength of 517 nm by antioxidant compounds. Firstly,100 µL of pomegranate sour was transferred into test tube having 3000 µL of DPPH solution. It was vortexed and kept for 30 min. The absorbances of the samples were then read at 517 nm in spectrophotometer device (Shimadzu UV-1800, Japan). Ascorbic acid was used as standards and the same procedure was applied for the standards (24). The total DPPH % inhibition and DPPH were calculated based on calibration graph of ascorbic acid (25, 50, 100, 150, 200, 250 µg/mL) and the values were expressed as ascorbic acid.

$ABTS^{\bullet+}$ radical scavenging capacity

The method is based on the reduction of $ABTS^{\bullet+}$ radical with antioxidant substances (25). Firstly, sour sample was

weighed as 150 µL and 2850 µL ABTS solution was added on it. The mixture was vortexed and kept in the dark for 120 min. Ascorbic acid was used as standard. Absorbance values of standards and all samples were measured at 734 nm spectrophotometrically (24). It was calculated as equivalent ascorbic acid. In addition, the % inhibition values of the samples were calculated.

Ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power method (FRAP), which is one of the most widely applied Iron (III) methods among the antioxidant capacity and activity methods, was developed by Benzie and Strain (26). Firstly, 250 µL of pomegranate sour sample and 2750 µL of FRAP solution were pipetted into same test tube, respectively. The solution was vortexed and kept for 30 min. A calibration graph was plotted using $FeSO_4$ (25, 50, 100, 200 and 400 µg/ml) standard. The total iron reduction antioxidant capacity was given as $FeSO_4$ equivalent (mg FeSO4 /kg) based on this graph (24). All measurements of the samples and standards were carried out at 593 nm spectrophotometrically.

Determination of total antioxidant capacity (TAC)

In the method, 500 µL of sample was pipetted into test tube having 2500 µL of distilled water and TAC molybdate solution (1000 µL) was added on the mixture and was vortexed. Then, the mixture was left incubation in water bath (95°C) for 90 min. In the method, the ascorbic acid was used as standard. The absorbance values of the mixtures and standards were read at 695 nm spectrophotometrically (27).

Total phenolic content (TPC)

It was developed by Singleton and Slinkard (28) to determine the total phenol content of the substances. In determination of phenolic content of the pomegranate sours were used folin-ciocalteau reagent. First, 300 µL of pomegranate sour sample was pipetted into test tubes and 3.4 mL of distilled water added. Then, 500 µL methanol and 200 µL folin–ciocalteu were transferred into the mixture and the mixture was vortexed, kept at the room temperature for 10 min. Finally, solution Na_2CO_3 (10%, 600 µL) was added, it was vortexed again. Then it was incubated in the dark at room conditions for 120 min. At the end of the period, the absorbance of the mixture was read at 760 nm. The results were determined in gallic acid equivalents (GAE) using the standard curve.

Total flavonoid content (TFC)

In this method, 500 µL of the sample and 3200 µL of methanol were transferred into the test tube and vortexed. Then 150 µL of both 0.5 M NaNO2 solution and 0.3 M $AlCl_3$ solution were pipetted onto the mixture and kept at the room temperature for 5 min. After that 1 mL of 1M sodium hydroxide solution added to mixture and it was kept for 10 min. Calibration graph was drawn based on catechin standard (25, 50, 100, 200, and 400 µg/mL) and amounts of total flavonoids were given as catechin equivalents CE mg/mL (27).

Statistical Analysis

The results of the physicochemical analysis of pomegranate sour samples and their antioxidant activity values were statistically evaluated with XLSTAT software (2010) included Principal Component Analysis (PCA), Duncan test.

RESULTS AND DISCUSSION

Physical and Chemical Parameters

Color is one of the most important quality parameter that affects the consumer's perception (29). L*, a*, b* and ΔE* color values of the pomegranate sour samples were given in Table 1. The lowest and highest L* values were measured as15.44 in the sample N3 and 20.48 in the sample N8, respectively. While the highest value of a* was determined as 14.17 (N16), the lowest value was measured as 8.22 (N1). In addition, the highest values b* and ΔE* was measured as - 0.47 (N7) and 6.19 (N8), while the lowest values were detected as -4.71 (N3) and 00.00 (N16), respectively. The differences between the colors values of the samples were found to be statistically significant ($p<0.05$). The main reason for differences in the color values of the pomegranate sours was the loss of water-soluble anthocyanins (form red color in the fruits) which are intensely found in the natural structure of the pomegranate fruit. In addition, another reason for the decrease in a* value can be attributed to the maillard reaction that occurs during heat treatment in pomegranate sours. Heat treatments show negative effects on the flavor, color and nutritional value of foods, in particular high heat treatments (30). Zaouay et al. (31) reported that color values of pomegranate juices produced from different pomegranate cultivars were varied from 51.7 to 83.9 in L*, 6.2 to 29.7 in a* and 7.1 to 23.7 in b*. In the other study, the L*, a* and b* values of pomegranate molasses, which are commercially available were reported as 1.88, 2.30, 2.39, respectively (32). The color values of pomegranate sours were varied in a wide range. The reason for this is both the cultivar type of pomegranate fruit used in the production and the applied heat treatment. However, it was determined that the ΔE* and a* values of the N16 pomegranate sour sample were statistically different ($p<0.05$) from the other pomegranate sour samples which

Table 1. Color values of pomegranate sour samples.

Sample	L*	a*	b*	ΔE*
N1	16.09±0.30^{cdef*}	8.22±0.29^{i}	-3.83±0.15^{bcd}	5.98±0.68^{a}
N2	16.65±0.43^{bcde}	8.96±0.23^{fgh}	-4.32±0.08^{cd}	5.30±0.65^{a}
N3	15.44±0.09^{f}	9.76±0.15^{de}	-4.71±0.04^{d}	4.49±1.10^{a}
N4	15.69±0.39^{ef}	9.47±0.13^{def}	-4.13±0.04^{ab}	4.19±1.10^{a}
N5	16.88±0.09^{bcde}	9.63±0.47^{de}	-2.94±0.24^{abcd}	4.78±0.48^{a}
N6	16.28±0.12^{cdef}	9.24±0.20^{efg}	-4.42±0.58^{cd}	4.96±0.72^{a}
N7	19.88±0.85^{a}	11.08±0.21^{a}	-0.47±0.44^{a}	6.17±1.15^{b}
N8	20.48±1.63^{a}	10.37±0.52^{bc}	-2.46±0.85^{abcd}	6.19±1.41^{b}
N9	17.21±1.13^{bc}	8.78±0.80^{ghi}	-4.04±0.23^{bcd}	5.63±0.28^{a}
N10	17.27±0.31^{bc}	8.51±0.13^{hi}	-3.91±0.33^{bcd}	5.81±0.91^{a}
N11	15.70±0.35^{ef}	9.23±0.15^{efg}	-4.47±0.24^{cd}	4.99±1.08^{a}
N12	15.94±0.59^{def}	9.23±0.48^{efg}	-4.27±0.82^{cd}	5.07±0.56^{a}
N13	16.42±0.01^{cdef}	10.43±0.0^{9bc}	-2.37±0.04^{abcd}	4.17±0.86^{a}
N14	17.74±0.08^{b}	9.88±0.37^{cd}	-1.88±0.63^{abc}	5.15±0.62^{a}
N15	16.95±0.30^{bcd}	8.78±0.10^{ghi}	-4.15±0.39^{bcd}	5.50±0.94^{a}
N16**	16.27±0.27^{g}	14.17±0.97^{ab}	-4.28±0.24^{d}	00.00±0.0^{a}

*Different letters means significantly different at p<0.05 according to Duncan test.
**Control sample

Table 2. Viscosity and shear stress values of pomegranate sour samples at 50 shear rate.

Sample	Shear Rate	Viscosity (mpa.s)	Shear Stress
N1	50	522.026^{e*}	528.06±2.91^{e}
N2	50	459.673^{ef}	462.97±1.423^{ef}
N3	50	690.803^{d}	687.11±1.125^{d}
N4	50	266.346^{f}	269.0±5.411^{f}
N5	50	542.982^{e}	546.92±1.785^{e}
N6	50	496.164^{ef}	516.03±2.325^{ef}
N7	50	333.685^{f}	330.72±1.806^{f}
N8	50	784.842^{c}	784.04±1.224^{c}
N9	50	544.067^{e}	517.01±1.237^{e}
N10	50	2158.625^{b}	2119.19±4.759^{b}
N11	50	246.293^{g}	274.14±0.917^{g}
N12	50	402.587^{ef}	385.92±3.039^{ef}
N13	50	50.699^{h}	52.446±2.326^{h}
N14	50	316.933^{f}	325±1.781^{f}
N15	50	4320.933^{a}	4087.6±3.235^{a}
N16**	50	368.536^{f}	378.025±1.285^{f}

*Different letters means significantly different at p<0.05 according to Duncan test.
**Control sample

were similar among themselves. In addition, the results of artificial dye indicated that it was not detected artificial dye in pomegranate sour samples.

Viscosity is a determinant of many factors that affect liquid performance during food processing, such as droplet breaking in spray drying, flow into molds and formability, pumpability, and emulsion formation. Particularly, it effects the quality of liquid food products in terms of appearance, stability and flavor release (33). The viscosity values of pomegranate sour samples at 50 shear rate were measured between 50.699 (N13)- 4320.933 mpa.s (N15) (Table 2). Differences in viscosity values of pomegranate sour samples may depend on brix and sucrose content of the sample, also on the applying temperature and time in the production. Therefore, while N15 sample had the lowest brix value and highest sucrose content, it also had the highest viscosity value. The same situation was present in the sample N10 (Table 3).

Determining the level of soluble solids (brix) in fruits and vegetables is important because it is an objectively measurable criterion used in the assessment of the taste or sweetness of foods (34). While the highest brix level was measured in the sample N15 (75.70%), the lowest brix level was determined in the sample N13 (59.20%). In the sample N16 (produced in laboratory), it was measured as 72.33 (Table 3). According to TS 12720 standard (traditional sour pomegranate concentrate) (14), brix values of pomegranate sour must be at least 68%. Although most of the sour samples were compatible with TS 12720 standard, the values of some samples (N12, N13 and N14) were detected below the minimum standard value (68%) and were statistically different (p<0.05) from the other samples. The differences in the brix

values can be related to the cultivar type of pomegranate fruit and the production process conditions.

Titratable acidity and pH are interrelated parameters that have a unique effect on food quality. However, titratable acidity is a better indicator of the effect of acid on flavor than pH (35). pH values of the samples were varied from 2.00 in sample N7 to 3.03 in sample N16 (Table 3). According to the TS 12720, the pH values of pomegranate sour should be between 2.4-4.0. The samples have pH values compatible with the standard except for the samples of N7, N8, N10, N11, N14 and N15. The lowest and highest titratable acidity (in citric acid) values of the samples were measured as 2.91(N15) and 8.75% (N16), respectively (Table 3). The minimum level of the titratable acidity (in citric acid) was declared as 6.0% (m/m) in TS12720 standard. The samples of N1, N3, N4, N6, N10, N13 and N15 were not compatible the standard. Poyrazoğlu et al. (36) reported that pH values of pomegranate juices prepared using 13 different varieties were varied from 3.29 to 3.93, also titratable acidity were measured between 4.58-17.30 g/L. In addition, in other study, the lowest and highest values of acidity (in citric acid) were reported as 8.3 and17.4 in 23 concentrated pomegranate juice samples, respectively (7). Acidity and pH values of the pomegranate sours may vary depending on the pomegranate varieties, environmental effects on growing, fruit ripeness and heat treatment methods.

The maillard reaction which plays an important role in improving their appearance, taste and aroma in foods occurs during heat treatment (37). However, maillard reaction products such as HMF, which are formed by the decomposition of sugars, are formed during heat treatment, especially also at high temperatures (38). It has been reported that HMF showed different effects on human health, such as carcinogenic, neoplastic transformation, and nephrotoxic, hepatotoxic and antioxidant activity and for this reason, it is important for food safety that the amount of HMF in foods is below the limit levels (39-40). The lowest and highest values of HMF were measured as 13.46 (N16) and 8117.66 (N14) mg/kg, respectively (Table 3). It was determined that there were statistically significant differences ($p<0.05$) between the HMF values of the pomegranate sour samples. According to TS 12720 (11) the maximum limit level of HMF in pomegranate sours was 50 mg/kg. Only, HMF level of samples N8 and N16 was detected below the limit value. Formation of high amounts of HMF is related to the direct heat treatment of pomegranate sour. Therefore, level of HMF in the control sample produced at high pressure and low temperature in a rotary evaporator was measured below the limit level.

The highest values of fructose, glucose, sucrose and total sugar content of the samples were measured as 43.36% (N4), 33.95% (N1), 39.58 % (N15), 73.76% (N3), respectively (Table 4). While the fructose, glucose were not detected in the sample N15. Also sucrose was not detected in all of the samples except the samples of N10 and N15. According to TS 12720 standard (11), traditionally produced pomegranate sour should not contain sucrose and amounts of glucose and fructose should be at least 20% (w/w) and17% (w/w), respectively. Therefore, N10 and N15 samples are not suitable for the TS 12720 standard. In addition, Zhang et al., (41) pointed out that presence of sucrose in pomegranate juice indicates that cane sugar was added to it and in addition high fructose syrups and invert sugars are other common sugar sources for adulteration. High sucrose concentration is the main parameters for the detection of adulteration in pomegranate (42).

Antioxidant Activity

Pomegranate fruits have a high antioxidant activity due to the including phenolic compounds such as flavonoids anthocyanins, tannins, phytoestrogenic and ellagic acid (43). It showed antioxidant properties similar to or higher than other foods such as red wine and green tea that are considered to have high antioxidant activity (44). Antioxidant activity values of the samples of N15 and N10 were determined at a very low level compared to the other samples in all antioxidant activity tests (Table 5-6). The values were also indicated that the samples of N10 and N15 were statistically different ($p<0.05$) from the other pomegranate samples. Many factors such as raw material, storage, high heat treatment, extraction and solvent type may be effective in the occurrence of low activity values in these samples. However, according to other analysis results, particularly, the high amount of sucrose in these samples indicates that the low activity values in these samples originated from raw materials. The other samples except for sample N10 and N15 showed high antioxidant activity in all antioxidant activity tests (Table 5-6).

Principal component analysis of the pomegranate Sour Sample (PCA)

Principal component analysis biplot (axes F1 and F2: 69.00 %) for the 16 pomegranate sour samples and their aggregation based on physical, chemical, antioxidant parameters were given Fig. 1. F1 and F2 plot explains 54.85% and 14.15%, respectively. Pomegranate sour samples formed into 4 groups. Particularly, samples of N10 and N15 (not comply with TS 12720) significantly were different from other groups. The samples N7 and N8, which form a separate group, were also differed from other samples only with their color analysis values.

Table 3. Brix, titratable acidity, pH, HMF content of the pomegranate sour samples.

Sample	Brix (%)	%Titratable Acidity (in citric acid)	pH	HMF mg/kg
N1	71.05±0.23^{de*}	5.88±0.24^{f}	2.72±0.003^{d}	746.89±28.83^{f}
N2	71.63±0.85^{cde}	6.88±0.32^{d}	2.51±0.004^{f}	222.09±9.35^{ij}
N3	73.40±0.26^{b}	5.18±0.11^{h}	2.83±0.02^{c}	406.64±13.06^{gh}
N4	72.07±1.07^{cd}	5.67±0.08^{g}	2.70±0.002^{d}	215.03±12.58^{ijk}
N5	69.77±0.35^{f}	6.17±0.03^{e}	2.70±0.02^{d}	5956.92±327.00^{b}
N6	71.93±0.32^{cd}	5.02±0.08^{h}	2.88±0.01^{b}	1728.87±97.25^{e}
N7	70.53±0.23^{ef}	6.83±0.06^{d}	2.00±0.01^{j}	355.30±19.13^{hi}
N8	73.80±0.44^{b}	7.83±0.01^{c}	2.13±0.01^{i}	33.48±1.44^{kl}
N9	70.97±0.40^{de}	6.36±0.04^{e}	2.63±0.01^{e}	558.07±32.73^{g}
N10	75.07±0.97^{a}	5.10±0.10^{h}	2.23±0.01^{h}	77.78±0.78^{jkl}
N11	71.20±0.20^{de}	8.60±0.002^{ab}	2.26±0.01^{h}	293.33±8.05^{hi}
N12	62.40±1.44^{h}	8.50±0.06^{b}	2.83±0.01^{c}	1899.38±59.98^{d}
N13	59.20±0.10^{i}	4.98±0.07^{h}	2.84±0.01^{c}	3932.40±18.97^{c}
N14	67.30±0.44^{g}	7.00±0.10^{d}	2.37±0.01^{g}	8117.66±207.51a
N15	75.70±0.26^{a}	2.91±0.04^{i}	2.03±0.06^{j}	73.645.59^{jkl}
N16**	72.33±0.10^{c}	8.75±0.06^{a}	3.03±0.06^{a}	13.46±0.02^{l}

*Different letters means significantly different at p<0.05 according to Duncan test.

**Control sample

Table 4. Glucose, sucrose and total sugar content of the pomegranate sour samples.

Sample	Fructose	Glucose	Sucrose % (m/m)	Total sugar
N1	37.34±0.39^{d*}	33.95±0.35^{a}	ND	71.27±0.05^{bc}
N2	38.31±0.27^{d}	31.83±0.41^{b}	ND	70.14±0.15^{c}
N3	43.04±0.5^{a}	30.72±0.58^{bcd}	ND	73.76±1.18^{a}
N4	43.36±1.17^{a}	29.10±0.69^{de}	ND	72.46±0.49^{ab}
N5	39.90±0.35^{c}	30.32±0.28^{bcde}	ND	70.22±0.63^{c}
N6	42.51±0.68^{a}	29.41±0.48^{de}	ND	71.92±0.20^{abc}
N7	41.08±0.39^{bc}	29.02±0.26^{de}	ND	70.11±0.64^{c}
N8	42.72±1.06^{a}	29.22±0.65^{de}	ND	71.94±0.41^{abc}
N9	42.06±0.92^{ab}	28.83±0.47^{e}	ND	70.89±1.39^{bc}
N10	1.38±0.05^{g}	0.18±0.01^{f}	24.15±1.49b	25.72±1.45^{h}
N11	42.7±0.65^{a}	28.91±0.51^{e}	ND	71.61±1.16^{bc}
N12	33.45±0.42^{e}	29.95±0.07^{cde}	ND	63.40±0.49^{e}
N13	29.49±1.97^{f}	30.45±0.98^{bcde}	ND	59.94±1.00^{f}
N14	37.28±0.43^{d}	29.71±3.22^{cde}	ND	66.98±2.79^{d}
N15	ND	ND	39.58±0.71a	39.58±0.71^{g}
N16**	40.84±0.39^{bc}	31.23±0.19^{bc}	ND	72.07±0.59^{abc}

*Different letters means significantly different at p<0.05 according to Duncan test.

**Control sample

Table 5. Antioxidant activity values (DPPH, DPPH % Inhibition, ABTS, ABTS % Inhibition) of the pomegranate sours.

Sample	DPPH mg AA/kg	DPPH % Inhibition	ABTS mg AA/kg	ABTS % Inhibition
N1	22185.96±143.14^{de}*	89.43±0.58^{e}	715.80±1.04^{bc}	98.42±0.15^{bc}
N2	22796.98±29.98^{ab}	92.20±0.12^{bc}	720.72±1.04^{ab}	99.43± 0.15^{ab}
N3	22328.06±25.42^{d}	92.23± 0.11^{bc}	707.33±0.59^{de}	99.66±0.08^{a}
N4	22729.46±14.86^{b}	92.72±0.06^{ab}	710.40±1.03^{cd}	98.85±0.15^{abc}
N5	22502.65±68.17^{c}	91.71± 0.28^{c}	703.20± 15.02^{de}	97.76±2.09^{c}
N6	22685.37±64.82^{b}	92.48± 0.27^{b}	710.56±1.19^{cd}	98.81±0.17^{abc}
N7	22237.51±53.83^{d}	90.31±0.22^{d}	706.72±2.60^{de}	97.90±0.36^{c}
N8	22333.56± 105.72^{d}	89.64± 0.4^{e}	703.87±3.67^{de}	96.37±0.50^{d}
N9	22680.22±91.10^{b}	90.55± 0.36^{d}	724,45±0.61^{a}	98.66±0.08^{abc}
N10	10.37± 0.004^{h}	0.04±0.003^{f}	15.90±0.60^{h}	2.20±0.08^{f}
N11	21813.39±14.61^{g}	90.48±0.06^{d}	690.89±1.54^{f}	97.75 ±0.22^{c}
N12	21967.24±38.97^{fg}	90.41±0.16^{d}	701.06±4.67^{e}	98.43±0.66^{bc}
N13	22069.53±155.94^{ef}	89.57±0.63^{e}	708.17±0.59c^{de}	98.04±0.09^{c}
N14	22916.19± 137.11^{a}	92.86±0.53^{ab}	715.16±1.04^{bc}	98.85±0.15^{abc}
N15	10.35±0.003^{h}	0.04±0.004^{f}	59.33±1.58^{g}	8.21 ±0.22^{e}
N16**	22647.55±206.21^{bc}	93.21±0.84^{a}	706.84±0.58^{de}	99.23±0.08^{ab}

*Different letters means significantly different at p<0.05 according to Duncan test.

**Control sample

Table 6. Antioxidant activity values (FRAP, TAC, TPC and TFC) of the pomegranate sours.

Sample	FRAP mg $FeSO_4$/kg	TAC mg AA/kg	TPC mg GAE/kg	TFC mg CE/kg
N1	5461.53±114.02^{bc}	6005.20±57.42^{c}	7399.08±127.03^{ab}	17306.53±878.75^{c}
N2	5306.23± 125.55^{ef}	5655.35±37.92^{d}	7636.82±28.96^{a}	23498.20±1500.30^{a}
N3	5043.32±134.17^{c}	5478.97±223.51^{de}	7667.89±118.43^{a}	16588.10±550.19^{cd}
N4	5623.85±292.54^{d}	5280.65±182.10^{e}	7720.29±49.74^{a}	21231.09±363.05^{b}
N5	4293.65± 115.55^{ab}	6787.03±278.20^{a}	6522.01±398.37^{c}	8828.84±99.70^{g}
N6	5734.43±71.19^{g}	6043.90±46.71^{c}	7570.89± 135.12^{a}	14851.36±470.54^{e}
N7	2424.52±145.14^{a}	4673.09±225.98^{f}	2320.83±209.54^{f}	3379.84± 458.51^{i}
N8	3619.32±99.68^{k}	3548.45± 34.66^{g}	4993.40±42.40^{d}	6600.66±114.78^{h}
N9	4049.02±172.07^{i}	6695.98±179.06^{ab}	6476.64±93.29^{c}	11232.81±176.27^{f}
N10	530.91±22.27^{h}	1276.91±9.92^{h}	1670.16±118.45^{g}	433.43±65.67^{j}
N11	4596.37±167.56^{l}	4563.55±132.19^{f}	7679.51±164.93^{a}	16241.29±417.24^{cd}
N12	4905.90±135.06^{f}	6862.62±76.02^{a}	7761.57±289.46^{a}	15829.93±1201.02^{de}
N13	2801.63±52.75^{de}	5316.82±40.17^{e}	3310.85±251.60^{e}	3775.07±511.88^{i}
N14	3985.86±47.86^{j}	6518.58±40.23^{b}	6681.64±425.26^{c}	7982.14±286.13^{g}
N15	593.04±2.20^{h}	1351.68±6.01^{h}	1577.95±235.05^{g}	3075.98±37.83^{i}
N16**	4705.09±49.08^{l}	5243.01±126.50^{e}	7159.09± 85.37^{b}	11521.29±502.06^{f}

*Different letters means significantly different at p<0.05 according to Duncan test.

**Control sample

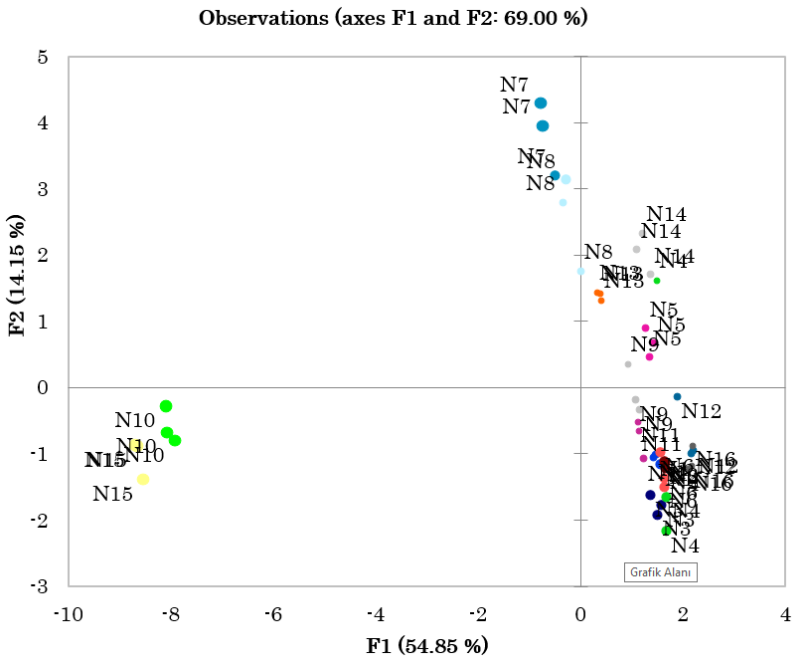

Figure 1. Principal component analysis of the pomegranate sour samples.

CONCLUSION

In this study, the physicochemical properties and antioxidant capacities of 15 traditionally produced pomegranate sold in the market and the one pomegranate sour sample produced in the rotary evaporator were investigated. In addition, the compliance of these pomegranate sour samples with the TS 12720 standard (11). were determined. It was detected that the pomegranate sours produced by the traditional method have different contents from each other. Firstly, the difference between the color values of the samples was found to be statistically significant ($p<0.05$) due to the loss of water-soluble anthocyanins, which are found in the natural structure of the pomegranate fruit. The viscosity values of pomegranate sour samples at 50 shear rate were measured between 50.699 (N13)- 4320.933 mpa.s (N15). The samples of N10 and N15 (%12.5 of all of the samples) did not show compatibility with TS 12720 (Anonymous 2016) due to contents of low glucose-fructose, high sucrose, high HMF, low pH and also low titratable acidity. Similarly antioxidant activity values of the samples of N15 and N10 were also determined at a very low level compared to at the all samples for all antioxidant activity tests. In addition, %25 of the samples with low pH, %43.75 of the samples with low titratable acidity, 18.75 of the samples with low brix and %87.5 of all the samples (except N8, N16) with high HMF were not conforming to the standard TS 12720.

Pomegranate fruit, pomegranate sours, pomegranate juice, and other pomegranate products should be consumed as food due to their protective and therapeutic effects against diseases. However, as in all traditionally produced other food products, pomegranate sour should be produced at the standard conditions and avoided adulteration.

CONFLICT OF INTEREST

Authors have declared no conflict of interest.

AUTHOR CONTRIBUTION

Authors declares the contribution of the authors is equal.

References

1. Shahidi F, Varatharajan V, Oh WY, Peng H. Phenolic compounds in agri-food by-products, their bioavailability and health effects. Journal of Food Bioactive 5 (2019) 57-119.
2. Kaur C, Kapoor HC. Antioxidants in fruits and vegetables–the millennium's health. International Journal of Food Science and Technology 36 (2001) 703-725.
3. Akar Z, Demir Ç, Alkan O, Can Z, Akar B. LC–MS/MS and RP–HPLC–UV analysis and antioxidant activities of Arum italicum Miller edible and nonedible tuber parts. Journal of Anatolian Environmental and Animal Sciences 6 (2021) 294-301.
4. Tsao R, Yang R. Optimization of a new mobile phase to know the complex and real polyphenolic composition: towards a total phenolic index using high-performance liquid chromatography. Journal of Chromatography A 1018 (2003) 29-40.
5. Özer CO, Var GB, Demir Özer E. Effects of extraction conditions on antioxidant activity and total phenolic content of pomegranate (Punica granatum) flower extracts. KSU Journal of Agriculture and Nature 24 (2021) 915-920.
6. Melgarejo-Sánchez P, Núñez-Gómez D, Martínez-Nicolás JJ,

Hernández F, Legua P, Melgarejo P. Pomegranate variety and pomegranate plant part, relevance from bioactive point of view: a review. Bioresour Bioprocess 8 (2021) 1-29.
7. Ekşi A, Özhamamci İ. Chemical composition and guide values of pomegranate juice, GIDA/The Journal of Food 34 (2009), 265-270.
8. da Silva JAT, Rana TS, Narzary D, Verma N, Meshram DT, Ranade SA. Pomegranate biology and biotechnology: A review. Scientia Horticulturae 160 (2013) 85-107.
9. Baysal T, Taştan Ö. Nar sağlıkta yıldız, in: Akçiçek, E., Kayalar H., Ötleş S., (Eds). Nar ürünleri ve üretimi. Gece Kitaplığı, İzmir, pp. 90-110, 2009.
10. Kavousi P, Mirhosseini H, Ghazali H, Ariffin AA. Formation and reduction of 5-hydroxymethylfurfural at frying temperature in model system as a function of amino acid and sugar composition. Food Chemistry 182 (2015) 164-170.
11. İncedayi B. Assessment of antioxidant properties and in-vitro bioaccessibility of some pomegranate products. Balıkesir Üniversitesi Fen Bilimleri Enstitüsü Dergisi 23(1) (2021) 96-110.
12. Kamış YE, Akar B, Baltacı C. Determination of physical, chemical and antioxidant properties of pomegranate sauces sold in Turkish markets. Turkish Journal of Analytical Chemistry 4(2) (2022). 67-75.
13. Turkmen, FU, Takci HM, Saglam H, Sekeroglu N. Investigation of some quality parameters of pomegranate, sumac and unripe grape sour products from Kilis markets. Quality Assurance and Safety of Crops and Foods, 11(1) (2019) 61-71.
14. Anonymous, Türk Standartlar Enstitüsü Nar Ekşisi Tebliği. Türk Standartlar Enstitüsü Ankara, 2016.
15. Quek SY, Chok NK, Swedlund P. The physicochemical properties of spray dried watermelon powders. Chemical Engineering and Processing: Process Intensification. 46 (2007) 386–392.
16. Tosun, İ., 1991. Standardı Olan Bazı Reçel Çeşitlerinin Bileşimi Üzerine Araştırmalar, Yüksek Lisans Tezi, Ondokuz Mayıs Üniversitesi, Fen Bilimleri Enstitüsü, 1991.
17. Cevik M, Tezcan D, Sabancı S, Icier F. Changes in rheological properties of koruk (unripe grape) juice concentrates during vacuum evaporation, Turkey. Academic Food Journal /Akademik Gıda 14 (2016) 322-332.
18. Anonymous, Çay-rutubet tayini. Türk Standartları Enstitüsü Ankara, 1990.
19. Anonymous, Meyve ve sebze ürünleri- titrasyon asitliği tayini. Türk Standartları Enstitüsü. Ankara, 2002.
20. Anonymous, Meyve ve sebze ürünleri- 5- hidroksimetilfurfural (5-hmf) içeriğinin tayini. Türk Standartları Enstitüsü, Ankara, 2002.
21. Baltacı C, Ilyasoglu H, Gundogdu A, Ucuncu O. Investigation of hydroxymethylfurfural formation in herle. International Journal of Food Properties 19 (2016) 2761-2768
22. IHC, 2009. International Honey Commission. (http://www.ihcplatform.net/ihcmethods2009.pdf. Retrieved June 10, 2021).
23. Brand-Williams W, Cuvelier ME, Berset CLWT. Use of a free radical method to evaluate antioxidant activity. LWT - Food Science and Technology 28 (1995) 25-30.
24. Ahmed D, Khan MM, Saeed R. Comparative analysis of phenolics, flavonoids, and antioxidant and antibacterial potential of methanolic, hexanic and aqueous extracts from Adiantum caudatum leaves. Antioxidants 4(2), 2015, 394-409.
25. Re R, Pellegrini N, Proteggente A, Pannala A, Yang M, Rice-Evans C. Antioxidant activity applying an improved ABTS radical cation decolorization assay. Free Radical Biology and Medicine 26 (1999) 1231-1237.
26. Benzie IFF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of 'antioxidant power': The FRAP assay. Analytical Biochemistry 239 (1996) 70-76.
27. Kasangana PB, Haddad PS, Stevanovic T. Study of polyphenol content and antioxidant capacity of Myrianthus arboreus (Cecropiaceae) root bark extracts. Antioxidants 4 (2015) 410-426.
28. Singleton V., Slinkard K. Total phenol analysis: automation and comparison with manual method, American Society for Enology and Viticulture 28 (1977) 49-55.
29. Francis FJ. Quality as influenced by color. Food Quality and Preference 6 (1995) 149-155.
30. Lasekan O, Ng S, Azeez S, Shittu R, Teoh L, Gholivand S., Effect of pulsed electric field processing on flavor and color of liquid foods. Journal of Food Processing and Preservation 41 (2017) e12940.
31. Zaouay F, Mena P, Garcia-Viguera C, Mars M. Antioxidant activity and physico-chemical properties of ind crops prod Tunisian grown pomegranate (Punica granatum L.) Cultivars. Industrial Crops and Products 40 (2012) 81-89.
32. Yilmaz Y, Çelik I, Isik F. Mineral composition and total phenolic content of pomegranate molasses. Journal of Agriculture, Environment and Food Sciences 5 (2007) 102-104.
33. Abbas KA, Abdulkarim SM, Saleh AM, Ebrahimian M. Suitability of viscosity measurement methods for liquid food variety and applicability in food industry-a review. Journal of Agriculture, Environment and Food Sciences 8 (2010)100-107.
34. Lister G, Tonsor GT, Brix M, Schroeder TC, Yang C. Food values applied to livestock products. Journal of Food Products Marketing 23 (2017) 326-341.
35. Sadler GD, Murphy PA. pH and titratable acidity. In: Nielsen SS (Ed.). Food analysis. Springer, Boston, pp. 219-238, 2010.
36. Poyrazoğlu E, Gökmen V, Artık N, Organic acids and phenolic compounds in pomegranates (Punica granatum L.) grown in Turkey. Journal of Food Composition and Analysis 15 (2002) 567-575.
37. Martins SI, Jongen WM, Van Boekel MA. A review of maillard reaction in food and implications to kinetic modelling. Trends in Food Science and Technology 11 (2000) 364-373.
38. Delgado-Andrade C, Seiquer I, Haro A, Castellano R, Navarro MP. Development of the maillard reaction in foods cooked by different techniques. intake of maillard-derived compounds. Food Chemistry 122 (2010) 145-153.
39. Baltacı C, Akşit Z. Validation of HPLC method for the determination of 5- hydroxymethylfurfural in pestil, köme, jam, marmalade and pekmez. Hittite Journal of Science and Engineering, 3 (2016) 91-97.
40. Lee CH, Chen KT, Lin JA, Chen YT, Chen YA, Wu JT, Hsieh CW. Recent advances in processing technology to reduce 5-hydroxymethylfurfural in foods, Trends in Food Science and Technology 93 (2019) 271-280.
41. Zhang Y, Krueger D, Durst R, Lee R, Wang D, Seeram N, Heber D, International Multidimensional Authenticity Specification (IMAS) algorithm for detection of commercial pomegranate juice adulteration. Journal of Agricultural and Food Chemistry 57 (2009) 2550-2557.
42. Nuncio-Jáuregui N, Calín-Sánchez Á, Hernández F, Carbonell-Barrachina ÁA, Pomegranate juice adulteration by addition of grape or peach juices. Journal of the Science of Food and Agriculture 94 (2014) 646-655.
43. Mousavinejad G, Emam-Djomeh Z, Rezaei K, Khodaparast MHH. Identification and quantification of phenolic compounds and their effects on antioxidant activity in pomegranate juices of eight Iranian cultivars. Food Chemistry 115 (2009) 1274-1278.
44. Johanningsmeier SD, Harris GK. Pomegranate as a functional food and nutraceutical source. Annual Review of Food Science and Technology 2 (2011) 181-201.